Noise-Induced Changes in Electrocochleography in the Fischer 344/NHsd Rat

Capstone Document

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Abstract

There is a growing body of evidence suggesting that cochlear de-afferentation can occur without accompanying outer hair cell (OHC) loss after noise exposure. Due to the high incidence of noise exposure in the United States, there is a need to develop assessments that can identify and monitor this de-afferentation in noise-exposed listeners. The current study was undertaken to investigate changes in the components of electrocochleography (EcochG) in the Fischer F344/NHsd rat in order to assess long-term cochlear de-afferentation from hazardous noise. Changes in the action potential (AP) and cochlear microphonic (CM) input-output (I/O) functions were measured after noise exposure. The CM/AP ratio was examined to account for between-test differences since electrode differences and OHC damage would impact each component. Noise-induced cochlear de-afferentation was predicted to result in reduction of AP amplitude without change in the CM amplitude, thus resulting in elevation of the CM/AP ratio. Fischer 344/NHsd rats were exposed to a narrowband noise to induce permanent (PTS) or temporary threshold shifts (TTS). After exposure, the AP and CM I/O functions were measured once every 4 weeks for 24 weeks in order to assess long term cochlear de-afferentation. Results showed that the AP I/O function was depressed at all levels in ears with PTS. The CM/AP ratios revealed depression of the AP at weeks 8-24, indicating possible cochlear de-afferentation. In ears that experienced TTS, a recruitment-like effect was seen in the

AP I/O function at 4 weeks, indicating OHC pathology. Subsequent AP decreases were seen over the 4-24 week testing period, indicating cochlear de-afferentation. This was also seen in the CM/AP ratio, which increased over time due to reduction in AP amplitudes. The I/O functions obtained from ears that had experienced TTS or PTS displayed patterns consistent with long-term cochlear de-afferentation. The findings indicate that components of EcochG are sensitive to possible de-afferentation of the cochlea after noise-exposure.

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Chapter 1: Introduction

Noise induced hearing loss (NIHL) is a significant issue worldwide (Heinrich & Feltens, 2006). Currently, approximately 12.8% of the American population from 20 to 69 years, which corresponds to 23 million people, is estimated to have NIHL (Mahboudi, Zardouz, Oliaei, Pan, Barzargan, & Djalilian, 2012). Concern about NIHL in children has recently increased and NIHL is estimated to be present in approximately 12.5% of children from ages 6 to 19 years of age (Niskar et al., 2001). According to the National Institute for Occupational Safety and Health (NIOSH) approximately 5 to 30 million Americans are exposed to hazardous levels of noise in the workplace annually (NIOSH, 2012). Excessive noise can cause adverse non-auditory effects including elevated blood pressure, sleep disturbances, stress and annoyance (Nelson, Nelson, Concha-Barrientos & Fingerhut, 2005). Federal guidelines for noise exposure in the workplace focus on the prevention of permanent threshold shifts (PTS) (OSHA, 1974; NIOSH, 1998) based on the assumption that temporary threshold shift (TTS) is associated with non-hazardous levels of noise exposure (Kujawa & Liberman, 2009). However, recent evidence suggests that primary neuronal degeneration is seen in ears that experienced a fully reversible threshold shift after noise exposure (Kujawa & Liberman 2006, 2009; Lin, Furman, Kujawa & Liberman, 2011). These results indicate that noise levels that were previously

assumed to be benign may be hazardous, and these exposures can lead to neuronal damage that can negatively impact auditory processing (Kujawa & Liberman, 2009).

Noise-induced Cochlear Damage

Noise exposure causes a broad set of physical changes in cochlear structures including the organ of Corti, neuronal terminals, spiral ligament, and stria vascularis (Henderson, Bielefeld, Harris & Hu, 2006; Ohlemiller, 2008; Wang, Hirose & Liberman, 2002). Mechanical trauma (Ahmad, Bohne & Harding, 2003; Talaska & Schacht, 2007) and oxidative stress can lead to cochlear damage underlying NIHL (Heinrich & Feltens, 2006; Ohlemiller et al., 1999; Yamane et al., 1995). Depending on the duration and intensity of the noise exposure, recovery after noise exposure may be complete, resulting in a TTS, or incomplete, resulting in a PTS (Kujawa & Liberman, 2009; Heinrich & Feltens, 2006). While the relationship between magnitude of threshold shift and noise exposure is complex and frequency dependent, in general, as the energy of the noise exposure increases, the magnitude of threshold shift increases (Hamernik, Qui & Davis, 2007; Qui, Hamernik & Davis, 2006). However, this is only seen in low intensity exposures below approximately 115-130 dB SPL. Above this critical level, the magnitude of NIHL abruptly accelerates due to the rupturing of the reticular lamina (Ohlemiller, 2008; Pourbakht & Yamasoba, 2003). Recovery from noise exposure occurs over approximately two to four weeks and asymptotes to permanent and stable levels within this time frame. Threshold shift that persists for four weeks or more after noise exposure is considered permanent (Miller, 1974). Overall, with noise parameters consistent across individuals, there is large variation between individuals in magnitude of resulting

threshold shift (TS) due to many factors, including; genetics, prenatal health, and environmental conditions (Ohlemiller, 2008).

The location of damage to cochlear structures after noise exposure relates to the frequency of the noise exposure due to the tonotopic organization of the basilar membrane (Talaska & Schacht, 2007). Most hair cell loss occurs approximately half an octave above the center frequency of a high frequency octave band noise exposure (Clark & Bohne, 1978; Harding & Bohne, 2007) and an upward shift in relation to the center frequency of the exposure band is typically seen (Cody & Johnstone, 1981; Wang et al., 2003). However, hair cell loss can to occur well above and below the noise exposure (Bohne & Harding, 2000; Harding & Bohne, 2007) and basal hair cells are more susceptible to damage from noise (Henderson et al., 2006; Talaska & Schacht, 2007). Outer hair cell (OHC) loss has frequently been linked to PTS and OHCs are typically the first structures to show acoustic related injury (Bohne & Harding, 2000; Chen & Fecther, 2003; Wang et al., 2003). However, there is a poor correlation between OHC loss and degree of PTS in many mammalian species (Chen, 2002; Chen & Fechter, 2003, Ohlemiller et al., 2000) and inner hair cell (IHC) loss is often better correlated with PTS (Nordman et al. 2000; Harding et al. 2002). OHC death can occur within 24 hours of noise exposure and significant loss can continue for at least two weeks (Wang et al., 2002). IHC loss after noise exposure is considerably smaller than OHC loss and generally occurs after OHC loss (Nordman et al. 2000; Harding et al. 2002).

After noise exposure, hair cell stereocilia can be broken, fused together or have broken tip links that lead to loss of structural integrity (Pickles, Osborne & Comis, 1987; Tsuprun, Schachern, Cureoglu & Paparella, 2003). Stereocilia damage and repair has

been associated with TTS (Patuzzi, 2002) and tip links can regenerate over the same time period as the return of mechanoelectrical transduction (Zha, Yamoah & Gillespie, 1996). However, high-level noise exposures can lead to irreversible damage to stereocilia of both IHCs and OHCs (Nordman et al., 2000; Wang et al., 2002). Buckling of supporting pillar cells has been linked to both TTS and PTS (Nordman et al., 2000; Wang et al., 2002). Pillar collapse may be partially reversible, but has been seen only in cases on PTS in some species (Wang et al., 2002). Disruption of pillar cells has been postulated to lead to OHC stereocilia becoming detached from the tectorial membrane, an insult that also may be reversible (Nordman et al., 2000). Damage to pillar cells also interferes with the impedance of basilar membrane vibration, thus disrupting vibration (Henderson et al., 2006). Exposure to high level noise can lead to significant swelling of the stria vascularis (Wang et al., 2002). While swelling can be temporary (Hirose & Liberman, 2003), the degeneration of intermediate cells and subsequent shrinkage of the stria vascularis is permanent (Hirose & Liberman, 2003; Wang et al., 2002). Changes in the stria vascularis can lead to short-term endocochlear potential changes, but these changes recover after most noise exposures (Hirose & Liberman, 2003; Ohlemiller & Gagnon, 2007). Shifts in the endocochlear potential do not appear to be a significant underlying pathology of TTS or PTS (Hirose & Liberman, 2003), and are most consistently linked to age-related pathology that occurs independently of NIHL (Gates et al., 2002; Gratton &Schulte, 1995; Mills & Schmeidt, 2004).

Noise-induced Changes in Spiral Ganglion Neurons

Noise exposure can also lead to damage to Type I afferent neurons and their peripheral processes, which may underlie TTS (Puel, Ruel, Gervais & Pujol, 1998; Pujol

& Puel, 1999; Roberston, 1983; Wang et al., 2002). This swelling and vacuolization of afferent terminals after noise exposure is due to glutamate excitotoxicity (Puel et al., 1998; Pujol & Peul, 2006). A large amount of glutamate is released by the IHCs during high levels of noise. These levels of glutamate in the synapses of spiral ganglion neuron (SGN) fibers can overstimulate the post-synaptic receptor cell bodies, leading to excitotoxicity (Henderson et al., 2006; Le Prell et al., 2007). Toxic concentrations of glutamate trigger entry of fluid into cells which leads to swelling and can lead to rupturing of cell membranes and degeneration (Le Prell et al., 2007). Noise-induced trauma to the dendritic terminals of the SGNs leads to synaptic uncoupling and loss of function (Puel & Pujol, 1999). After noise- or drug-induced glutamate excitotoxicity, cochlear synaptic ultrastructure and auditory thresholds can recover, suggesting repair or regeneration (Puel et al., 1998, 1995; Zheng et al., 1997).

After hair cell loss due to noise exposure, SGNs can undergo secondary degeneration, especially in regions with destroyed IHCs (Stankovic et al., 2004; Sugawara, Corfas & Liberman, 2005; Zimmerman et al., 1995). However, primary loss of cochlear fibers does not lead to hair cell or supporting cell loss (Stankovic et al., 2003). The degree of degeneration of SGNs after IHC loss is variable (Sugawara et al., 2005) and can occur within weeks or months after noise exposure (Talaska & Schacht, 2007). Long-term survival of neurons is enhanced by the presence of intact supporting cells (Stankovic et al., 2004; Sugawara et al., 2005). Supporting cells ensheath the unmyelinated portion of the SGNs near the hair cell synapse and express many markers similar to the glial cells in the central nervous system that provide necessary trophic support (Rio et al., 2002; Stankovic et al., 2004). Neurotrophins BDNF and NT-3 can be

critical to the survival of SGNs, and are normally released by both hair cells and supporting cells (Stankovic et al., 2004; Sugawara et al., 2007). Interruption of a signaling pathway in the supporting cells that reduces expression of NT-3 leads to massive loss of SGNs without loss of IHCs (Stankovic et al., 2004). The difference in degree of SGN degeneration based on the presence of hair cells and supporting cells is consistent with the dependence of neuronal survival on neurotrophin release (Makary, Shin, Kujawa, Liberman & Merchant, 2011). In regions of IHC loss, degeneration of peripheral axons was first seen at 1 week after noise exposure and degeneration of cell bodies was seen 8 weeks after noise exposure (Wang et al., 2003).

Since hair cell loss occurs directly after noise exposure and SGN degeneration follows a much longer time course, this degeneration was believed to occur secondarily to hair cell loss (Kujawa & Liberman, 2009; Stankovic et al., 2004). However, recent evidence suggests that primary SGN loss can occur in the absence of hair cell loss over a period of several months to years after noise exposure that induced TTS (Kujawa & Liberman, 2006, 2009; Lin et al., 2011). Kujawa and Liberman (2006) exposed subsets of CBA/CaJ mice between the ages of 4-124 weeks to a 100 dB SPL 8-16kHz octave band noise for two hours while age-matched controls remained unexposed. Animals were then allowed to survive until 2, 16, 32, 64, or 96 weeks after noise exposure in order to examine the relationship between noise exposure and aging. Auditory brainstem response (ABR) thresholds and distortion product otoacoustic emissions (DPOAEs) were recorded before acoustic exposure and before harvest of cochlear tissues at the varying post-exposure intervals. Initial noise-induced threshold shifts were seen in both ABR and DPOAE measures at 2 weeks in young animals while older animals showed no

significant threshold shifts. However, at 96 weeks, ABR threshold shifts were seen across all test frequencies while insignificant changes in DPOAEs occurred. This suggests that the later threshold shifts were due to IHC and/or auditory nerve pathology and not due to progressive OHC damage. In ears that were exposed and then allowed to age, substantial loss of the SGNs was seen throughout the cochlea without substantial OHC or IHC loss. Since this also occurred in older mice that has only sustained noise-induced TTS, it suggests that neural degeneration can occur after an exposure that had previously been believed to be fully reversible (Kujawa & Liberman, 2006).

Primary degeneration of SGNs after noise-induced TTS was also seen after the same 100 dB SPL octave band acoustic exposure in 16 week old mice (Kujawa & Liberman, 2009) and in guinea pigs after exposure to a 4 to 8 kHz octave band at 106 dB SPL (Lin et al., 2011). These exposures resulted in a TTS that was larger in the ABR and electrocochleography (EcochG) action potential (AP) thresholds than in DPOAE elevation, suggesting that neural damage heavily contributed to the TTS (Kujawa & Liberman, 2009; Lin et al., 2011). ABR and DPOAE thresholds had recovered ten days after exposure in guinea pigs (Lin et al., 2011) and by two weeks in mice. Thresholds were stable from 8 to 16 weeks post exposure (Kujawa & Liberman, 2009). While amplitudes of the DPOAEs recovered at all test frequencies, ABR amplitudes recovered to only 40-50% of pre-exposure values (Kujawa & Liberman, 2009; Lin et al., 2011) and AP to only 60% of pre-exposure values out to 64 weeks after noise exposure suggesting neuronal loss despite OHC recovery (Kujawa & Liberman, 2009). Neither IHC nor OHC loss was seen in any exposed ears (Kujawa & Liberman, 2009; Lin et al., 2011). However, in the basal half of the cochlea there was substantial degeneration of pre-

synaptic and post-synaptic afferent auditory nerve structures in mice (Kujawa & Liberman, 2009). In the mammalian cochlea, typically a single type I ganglion neuron makes direct synaptic contact with a single IHC through a single terminal swelling (Kiang et al., 1982; Nayagam et al., 2010; Sugawara et al., 2005). At the end of the terminal swelling is a synaptic complex that consists of one pre-synaptic ribbon within the IHC that is believed to play a role in vesicle delivery (Liberman, 1980). Damage to these presynaptic ribbons was detected at all post-exposure times including 24 hours (Kujawa & Liberman, 2009) and at 10 days post exposure when ABR thresholds had recovered but amplitude had not (Lin et al., 2011). This early presynaptic ribbon damage occurred along with a corresponding proportion of the postsynaptic afferent terminal loss (Kujawa & Liberman, 2009). While loss of peripheral terminals was detected in mice soon after noise exposure, death of the cell bodies was very slow. Ganglion cell numbers were close to normal at 2 weeks after noise exposure, but one year after noise exposure, dramatic loss of SGNs was seen. The population of SGNs had decreased by approximately 50% by 2 years after exposure (Kujawa & Liberman, 2009). However, the close agreement between acute loss of terminals and delayed loss of cell bodies suggests that within 24 hours after noise exposure, the chain of events that lead degeneration of neural components had already begun (Kujawa & Liberman, 2009). In guinea pigs, however, only a small but statistically significant loss of SGNs was measured at two years in the basal half of the cochlea where there was maximal ribbon loss (Lin et al., 2011). This indicates that SGN loss may not match the degree of synaptic degeneration until later in these animals' lifespans or that certain species are more susceptible to SGN loss.

AP and ABR thresholds are insensitive to diffuse loss of IHC or SGNs as long as the cochlear amplifier is still functioning (Liberman et al., 1997). Diffuse loss of fibers does not impact threshold since the number of neuronal fibers that respond grows rapidly close to threshold (Taberner & Liberman, 2005). Theoretically, a 5 dB SPL increase in stimulus level could account for this neuronal loss, which will not be detected due to the step size and variability seen in ABR testing (Lin et al., 2011). Lin et al. (2011) also propose an additional hypothesis based on the differences in spontaneous rates of different subtypes. Different subtypes of type I afferent fibers exist based on differences in spontaneous rate and threshold sensitivity. In most species, auditory nerve fibers that have higher spontaneous rates have lower thresholds while those with low spontaneous rates have higher thresholds (Liberman, 1978; Taberner & Liberman, 2005). There appears to be a clear cut segregation of high and low threshold fibers on the pillar or the modiolar surfaces of the IHCs respectively (Liberman et al., 2011; Lin et al., 2011). If only high-threshold subtypes were lost after noise exposure, then the AP or ABR threshold should remain unchanged but the amplitude of supra-threshold measures would be reduced. However, morphological data from the guinea pigs did not reveal any differences in damage of ribbon loss on pillar and modiolar sides of the IHC (Lin et al., 2011), so this hypothesis has not been confirmed.

Loss of SGNs has been detected in humans, and has been attributed to part of the aging process. In human temporal bones from newborns to 100 years of age with no significant OHC or IHC loss, SGNs were calculated to decline at a mean rate of 100 per year (Makary et al., 2011). Precise timing and synchrony of neural firing is important for the accurate representation of temporal aspects of speech (Anderson et al., 2011). Age-

related hearing loss with evidence of primary neural degeneration is manifested as increased difficulty with speech intelligibility in acoustically complex environments that is disproportionately poor for the degree of threshold shift (Pauler et al., 1986; Schukneckt, 1993).

Noise exposure can lead to cochlear de-afferentation in mammalian models, and most likely in the human ear (Lin et al., 2011). In addition to the aforementioned difficulties of auditory processing of complex signals, peripheral neurodegeneration can lead to reorganization of the auditory cortex. Cortical regions that are deprived of input become occupied by representations of remaining cochlear loci (Irvine, Rajan & McDermott, 2000). These cortical reorganizations may lead to tinnitus and loudness sensitivity that may occur in the absence of threshold shift (Bauer et al., 2007; Kujawa & Liberman, 2009). Standard audiometric assessment is not sensitive to cochlear de-afferentation due to the fact that thresholds are insensitive to SGN loss (Liberman et al., 1997; Taberner & Liberman, 2005). Due to the high incidence of noise exposure, there is a need to develop assessments that can identify and monitor this de-afferentation in noise-exposed listeners before it leads to long-term auditory processing deficits.

Electrocochleography

EcochG reflects the electrical activity of cochlear structures. The AP wave represents far-field summed synchronous activity of the auditory nerve (Chertoff, 2004; Kujawa & Liberman, 2009). While at low intensities, the contributing neurons to the near-field compound action potential (CAP) that is recorded from the round window of the cochlea have characteristic frequencies nearly equal to the stimulus frequency. However, at high intensities an overlap in the population of contributing fibers to the CAP has been

observed in some models of round-window recordings and basal cochlear regions contribute to the response for both high and low frequency stimuli (Chertoff, 2004). Degeneration of SGNs leads to reduction in amplitude of CAP in addition to number of contributing fibers to the CAP (Lichtenhan & Chertoff, 2008). Noise-induced PTS can impact the number of auditory fibers that contribute to the CAP and also lead to steeper slopes in the number-level functions at higher frequencies (Chertoff, Lichtenhan, Tourtillot & Esau, 2008). Noise-induced PTS also results in steeper slopes in CAP input-output functions (Elberling & Salomon, 1976; Salvi et al., 1983). The increase in slope suggests a recruitment-like effect, which can result from OHC damage and broadening of the tuning of the basilar membrane leading to excitation of auditory fibers from outside regions (Chertoff et al., 2008).

The cochlear microphonic (CM) is generated from the spatial summation of current passing through OHCs in response to motion of the basilar membrane. The CM reflects the mechano-electric transduction process of the OHCs via deflection of the stereocilia (Chertoff, Amani-Taleshi, Guo & Burkard, 2002; Chertoff, Steele, Ator & Bian, 1996). The CM is dominated by OHC receptors on the linear tails of the excitation pattern (Cheatham et al., 2011). The CM is proportional to the displacement of the basilar membrane, and gain of the mechano-electric transduction reflects the amount of current passing through OHCs for a given displacement of the basilar membrane (Chertoff, Steele & Bian, 1997; Chertoff, Yi & Lichtenhan, 2003). The amplitude of the CM is not significantly impacted when OHCs are non-functional, such as in prestin knock-out mice, but is reduced when OHCs are absent (Liberman et al., 2002). Reductions in CM amplitude occur in animals with hearing loss due to the reduction of the amount of

current passing through OHCs (Chertoff et al., 1997; 2003). After noise exposure that induces PTS, the amount of current passing through the OHCs decreased dramatically with increasing noise-induced AP threshold shift due to hair cell loss and the reduction of the cochlear amplifier (Chertoff et al., 2003). After noise exposures inducing TTS, reduction of OHC current was significantly less than what was seen after PTS (Patuzzi, 1998) due to the lack of hair cell loss and changes to cochlear metabolic activities (Chertoff et al., 2003).

EcochG is clinically used in the diagnosis of Ménière's disease. Common characteristics seen in ears with endolymphatic hydrops are enhanced summation potential (SP) to AP ratio, increased AP latency difference between condensation and rarefaction clicks and broadened AP waveforms (Ohashi, Nishino, Aria & Koizuka, 2013). However, elevation of absolute amplitude of SP is not always found in patients with Meniere's and incidence of an elevated SP in the Meniere's population is 55-75% (Chung, 2004; Takeda & Kakigi, 2010).

Current Study

The current study investigated if the components of EcochG were sensitive measures for the identification and monitoring of cochlear de-afferentation after exposure to hazardous noise. The study utilized conventional AP I/O functions, and a novel application of EcochG, the CM/AP ratio. Measuring input-output (I/O) functions of the CM and AP allows assessment of the OHCs and the cochlear afferent pathway from the IHCs. Decreases in AP amplitude in the absence of changes of other cochlear potentials indicate that the afferent nerve fibers have been damaged in absence of hair cell pathology (Puel, 1995). AP I/O functions were predicted to show a decrease in amplitude

at all levels in cases of SGN pathology while OHC pathology would result in a recruitment-like effect and depression of responses to only low-level probes would be seen. The CM/AP ratio at different stimulus levels was examined to account for between-test differences since electrode differences and OHC damage would impact each component. Noise-induced cochlear de-afferentation was predicted to result in reduction of AP amplitude without change in the CM amplitude, thus resulting in elevation of the CM/AP ratio.

Chapter 2: Methods

Subjects

Eleven Fischer 344/NHsd rats were used in the study. Animals were obtained from Harlan Laboratories at 3-4 months of age and were housed in the Ohio State University Laboratory Animal Resources colony. All procedures involving use and care of the animals were reviewed and approved by The Ohio State University's Institutional Animal Care and Use Committee.

Electrophysiology

In order to assess AP and CM I/O functions and AP thresholds, free field EcochG was used. For all testing procedures, the rats were anesthetized with inhalant isoflurane (4% for induction, 1.5% for maintenance, 1 L/min oxygen flow rate). Induction was reached in a plastic induction box. After animals were anesthetized, they were moved for testing to a single-walled sound attenuation booth (Industrial Acoustics Company, Bronx, NY) in which anesthesia was delivered through a nose cone. Needle electrodes (Grass Technologies, West Warwick, RI) were placed behind both pinnae (non-inverting for ipsilateral responses, inverting for contralateral responses) and above the left hind leg (ground). Stimuli were generated using Tucker Davis Technologies (TDT, Gainesville, FL) SigGenRZ version 5.1 software. Tone bursts were employed at frequencies of 5, 10 and 15 kHz 1 msec in duration with a 0.5 msec rise/fall time with no plateau. Signals

were routed tospeaker (TDT Model MF1) positioned at 90 or 270 degrees azimuth, 3 cm from the ear being tested. The evoked responses were amplified with a gain of 50,000 through use of a headstage (TDT RA4LI) connected to a preamplifier (TDT RA4PA). Responses were bandpass filtered. In order to preserve the CM, the high-pass frequency remained at 100 Hz and the low-pass was adjusted to 10, 15, or 30 kHz for 5, 10 and 15 kHz tone burst stimuli, respectively.

For threshold and AP and CM I/O function testing, AP and CM were elicited with test stimuli consisting of non-alternating phase tone bursts. Eight hundred sweeps were averaged at each level of the stimulus using TDT BioSigRZ version 5.1. The stimuli were decreased from 90 dB SPL in 5 dB steps to 5 dB SPL or a level that was at least 10 dB below the lowest level that a response could be detected. Ears were tested separately, and both ears were tested in the same session, with the right ears assessed first. AP threshold was defined as the lowest level at which a detectable response could be elicited. Latency and amplitude of the AP were measured with amplitude assigned as the peak-to-peak amplitude from the positive peak to the following negative peak. Amplitude of the CM was assigned as the peak-to-peak amplitude from the highest peak to the lowest neighboring negative peak. Amplitudes at different stimulus levels were assembled into I/O functions.

Noise Exposure

Following baseline EcochG recordings, each animal was exposed to a 115 dB SPL narrowband noise centered at 15 kHz with a bandwidth of 10 Hz for thirty minutes. The noise was created using TDT RPvdsEX version 7.1. The noise was generated using a real time signal processor (TDT RP2) then amplified by a power amplifier (Marathon DJ-

5000, Marathon Professional, New York, NY) and delivered to a speaker. In order to achieve different exposures for each ear, each animal was anesthetized for the exposure using the same procedures used for EcochG testing. The speaker was placed 3 inches from the right ear, and the 115 dB SPL level reflects calibration at the level of the right ear. In addition to the head shadow effect, the left ear was occluded with a sound-attenuating plug. The total attenuation was approximately 25 dB for each rat. The noise levels were calibrated at the level of the animal's head using a sound level meter (LxT1, Larson Davis Inc., Depew, NY) and a ½" condenser microphone (Model 377B02, PCB Piezotronics, Inc., Depew, NY). After the noise exposure, EcochG was retested at 5, 10 and 15 kHz, once every 4 weeks for 24 weeks.

Statistical Analyses

For statistical analyses, the left and right ears were analyzed separately. Threshold data were analyzed using a two-factor repeated measures ANOVA (week x frequency). Subsequent analysis of the two-way interaction for the right ear threshold data was completed using one-way repeated measures ANOVAs (week) at each frequency. Both left and right ear threshold data were analyzed using a series of paired t-tests at each time point. Due to a significant main effect of test week, left ear threshold data were collapsed across frequency for this analysis.

Due to a large threshold shift at 15 kHz probes and variability in data at 5 kHz, only 10 kHz amplitude data were entered in the statistical analysis of I/O functions. Amplitudes of the AP and CM were converted from nanovolts to dB before inclusion in statistical analysis. For AP and CM/AP I/O functions, the curves were analyzed with two-factor repeated measure ANOVAs (probe level x week). Two-way interactions of probe

level and test time were analyzed using a series of student's t-tests. A p-value of <0.05 was considered significant in all analyses except paired samples t-tests to which the Bonferroni correction was applied.

Chapter 3: Results

As can be seen in Figure 1, right ears that were exposed to a 115 dB SPL narrowband noise experienced a mean threshold shift at 15 kHz of 40 dB SPL 4 weeks after noise exposure, with threshold shifts of 14.1 and 15.9 at 5 and 10 kHz, respectively. Statistical analyses revealed a two way interaction of week and frequency and a main effect of week at 5, 10, and 15 kHz. At 5, 10, and 15 kHz, pre-exposure thresholds were significantly different from all subsequent time points post-exposure. At 5 kHz, thresholds at weeks 4 and 8 were significantly different from thresholds at 16 weeks. At 10 kHz, thresholds at week 4 were statistically different from thresholds at 12, 16, and 24 weeks and week 16 thresholds were different from 24 weeks. Statistical analysis of left ear thresholds revealed no significant interaction, but main effects of both week and frequency. Pre-exposure thresholds across frequencies were different from thresholds at week 20 and 24. Week 4 and 8 thresholds were different from thresholds at 24 weeks.

Figure 2 displays the AP I/O functions for right and left ears with a 10 kHz probe before exposure and at each 4 week testing interval. Statistical analyses of AP I/O functions for the right ears showed main effects of week and level. Pre-exposure amplitudes were statistically different from amplitudes obtained at all other time points. Amplitudes at week 4 were significantly different from all other following time points at probe levels from 55 to 90 dB SPL. No significant differences were detected between

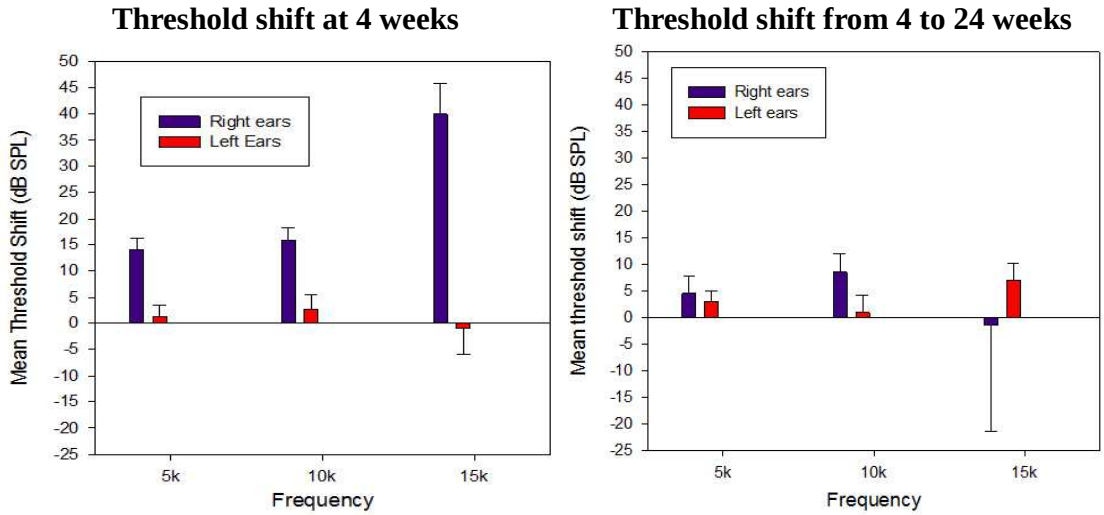

Figure 1: Threshold shifts in left and right ears at four weeks after noise exposure and the difference in thresholds from four to twenty-four weeks.

amplitudes from 8 to 24 weeks. Statistical analysis of the AP I/O of the left ears revealed a level by week interaction. Pre-exposure AP amplitudes at all probe levels above 45 dB SPL excluding 65 dB SPL were significantly different from all other time points except for at 4 weeks. Four-week recordings were not different from pre-exposure amplitudes at 90 and 80 dB SPL probe levels. Probe levels from 65 to 80 dB SPL at 4 weeks were greater than those from 8 to 24 weeks. No significant differences were found between recordings from 8 to 24 weeks.

CM/AP ratios at 10 kHz in right and left ears from 70 to 90 dB SPL probes from pre-exposure and at each 4 week testing interval are displayed in Figure 4. Levels below

10 kHz AP I/O function for Right Ears **10 kHz AP I/O function for Leftt Ears**

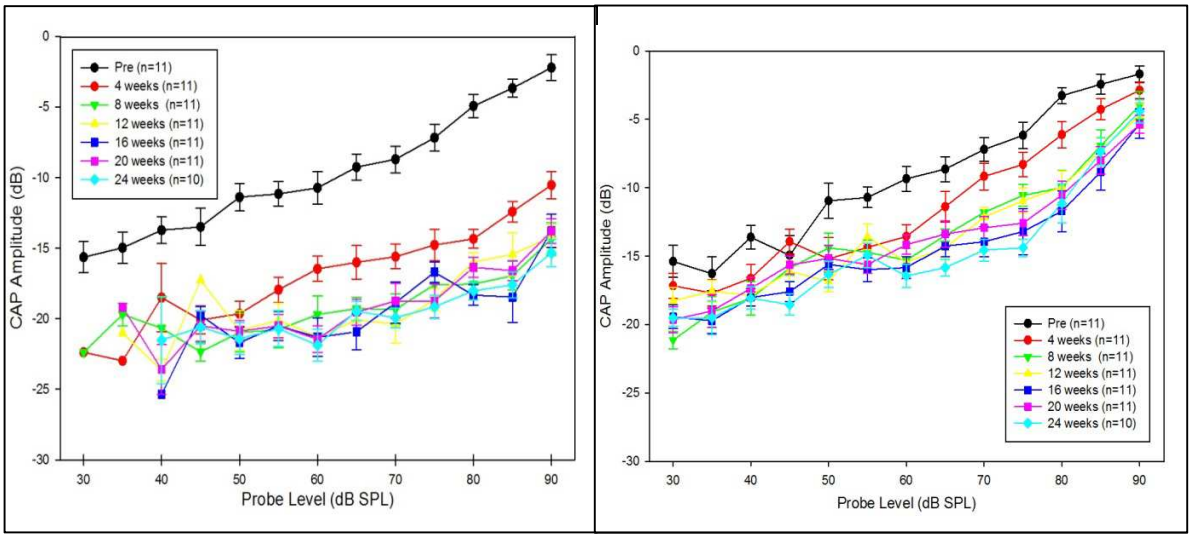

Figure 2: AP I/O functions collected from the right and left ears before and at four-week intervals after noise exposure. A PTS was induced in the right ears and a TTS in the left ears. Data were averaged across all animals. AP amplitude in dB was calculated with a reference to 1 nanovolt

70 dB SPL are not displayed due to the lack of recordable CMs below this level. Statistical analyses of the right ears revealed significant main effects of level and week. Pre-exposure and 4 week values were not statistically different and were lower than values obtained from 8 to 24 weeks. CM/AP ratios at 8, 12, 16, and 24 weeks were not statistically different from each other. The CM/AP ratio at 20 weeks was statistically different from measures at 8 and 12 weeks but not from 16 and 24 weeks. For CM/AP functions in the left ears, statistical analyses revealed main effects of week and level. CM/AP ratios at Pre and 4 weeks were not significantly different and ratios at 90 dB SPL

at all time points did not show any significant differences. Pre-exposure and 4 week CM/AP ratios were lower than ratios recorded at 8, 12 and 16 weeks except at 70 and 75 dB SPL probes and lower than 70-85 dB SPL measures taken at 20 and 24 weeks.

10 kHz CM/AP Ratio in Right Ears **10 kHz CM/AP Ratio in Left Ears**

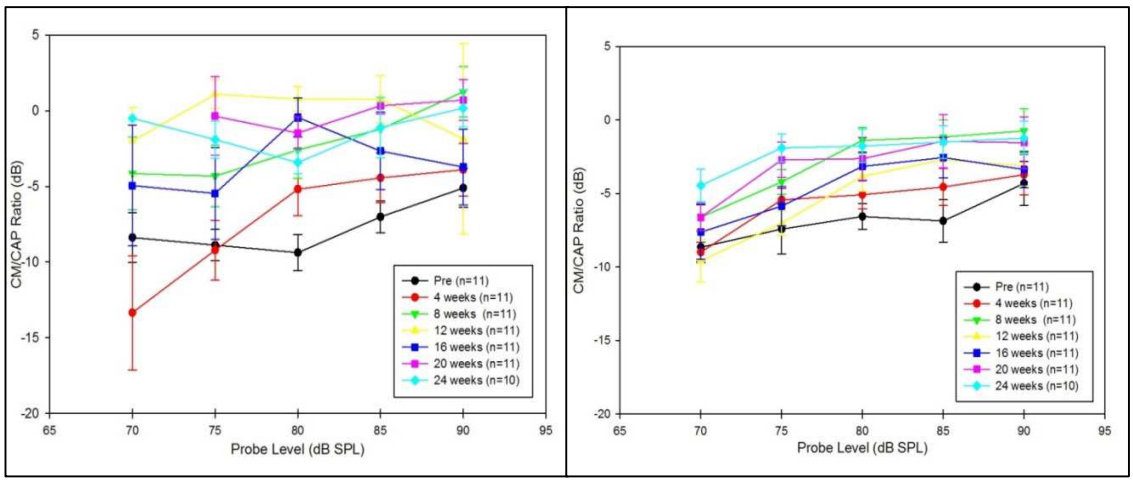

Figure 3: CM/AP I/O functions for right ears that experienced PTS and for left ears that experienced a TTS pre- and at four-week intervals post noise exposure . Data were averaged across ears. CM/AP amplitude in nanovolts was converted to dB using a reference of 1 nanovolt.

Chapter 4: Discussion

While the evidence supporting primary neural degeneration after TTS is currently limited, the current study provides evidence that physiologic changes consistent with cochlear de-afferentation can occur after noise exposure that induces threshold shift. Exposure to a 115 dB narrowband noise centered at 15 kHz induced a mean threshold shift of 40 dB at 15 kHz 4 weeks after noise exposure. This large AP threshold shift at 15 kHz obscured the results obtained from 15 kHz probes due to not only the shift of AP but the elimination the CM at most probe levels rendering the CM/AP un measureable. This exposure induced only a 15 dB threshold shift of AP at 10 kHz and 5 kHz. However, AP I/O functions for these right ears showed a large depression of AP amplitude at 10 kHz of approximately 70-80% at most levels 4 weeks after noise exposure. At this same time point, CM/AP ratios were unchanged from pre-exposure measures since the large decrease in AP amplitude was accompanied by a decrease in CM amplitude of the same magnitude. The depression of both components suggests that the decrease of AP amplitude was in part due to the lack of gain provided by the OHCs. Neural damage or IHC loss most likely contributed to the decrease in AP amplitude at this time, since OHC loss in isolation would be expected to only impact low-level signals, as was seen in the left ears that experienced TTS. Depression of amplitude at all levels suggests neuronal and/or IHC damage, but the threshold shift rendered a definitive conclusion impossible to

make on the basis only of the AP I/O functions. Subsequent decreases in amplitude of the AP from 4 weeks to 8 weeks in ears that experienced PTS are consistent with subsequent cochlear de-afferentation since hair cell loss after noise exposure does not continue past 4 weeks. Due to the degree of PTS observed after noise exposure, it is likely that both IHC and OHC loss occurred in addition to damage to supporting cells. Subsequent degeneration of SGNs in the current study is consistent with previous findings that SGN survival is dependent on the neurotrophic support provided by IHCs and supporting cells (Stankovic et al., 2004; Sugawara et al., 2007). These decreases in AP plateaued at 8 weeks suggesting subsequent damage to SGNs did not occur further into the 24 week time span.

In the left ears that experienced only TTS, 4 weeks after noise exposure AP I/O functions showed a recruitment-like effect in that the AP amplitude at low levels was depressed, but amplitude at high level probes was preserved. Steeper I/O functions of the AP after noise exposure is consistent with OHC pathology. The CM/AP ratio was unaffected at this time, which was expected with the assumed damage to the OHCs. At 4 weeks after noise exposure, the amplitude of the AP was approximately 20-30% from pre-exposure measures at levels above 50 dB whereas the CM was reduced by 5-15% at levels above 70 dB. The CM/AP ratio most likely was unchanged despite the larger AP reductions due to the fact that the data were converted to dB, which could have obscured these changes from being statistically significant. The amplitude of the CM may have been relatively unaffected due to the lack of frequency specificity of the CM and off-frequency contributions compensated for OHC loss that may have impacted the AP at 10 kHz. Additionally, there may have been a population of remaining OHCs that was able to

generate the CM despite loss of OHCs contributing to the reduction of cochlear gain. After 4 weeks, subsequent decreases in AP amplitude of approximately 50% at all levels above 50 dB SPL were detected at 8 weeks, and these amplitudes were stable at all other time point until the end of testing. This decrease between 4 and 8 weeks at all levels is consistent with damage to SGNs or IHCs. As previously discussed, hair cell loss after noise exposure typically occurs soon after noise exposure and would not be expected to occur after 4 weeks. Therefore this decrease in AP amplitude suggests cochlear de-afferentation. Degeneration of SGNs occurs in areas that experience IHC loss (Stankovic et al., 2004; Sugawara et al., 2005; Zimmerman, 1995). However, since IHC loss most likely did not occur, this suggests that damage to the cochlear afferent fibers was due to a different underlying mechanism than loss of trophic support. The decrease in amplitude of AP was reflected in the CM/AP ratio which was significantly higher at testing from 8-16 weeks due to reduction of the AP but stable CM recordings. Additionally, subsequent increases in the CM/AP ratio at 20 and 24 weeks occurred despite no significant changes in AP in this time period. This suggests that the CM was elevated at both of these time periods relative to previous testing times or there was subsequent cochlear de-afferentation to which the AP alone was insensitive. Data collected in a separate study on a group of rats exposed to a sub-clinical exposure of the same narrow-band noise centered at 15 kHz and delivered at a level of 70 dB SPL did not show any significant changes over time due to age or noise. However, AP I/O functions showed aberrant elevation at 16 weeks despite stability of CM/AP ratios at all time points. This suggests that CM/AP ratios are not impacted by between test differences, and measured

fluctuations due to recording anomalies impact the CM and AP equally leading to a lack of change of the CM/AP ratio.

NIHL and age related hearing loss commonly co-exist and impact similar cochlear structures (Ohlemiller, 2008). Rats were 3-4 months at the beginning of testing, thus were only 9-10 months at the end of testing and harvesting of cochlear tissues. Thresholds in F344 rats show relatively little change over the first year of life, but are dramatically decreased by 18-24 months of age (Bielefeld et al., 2008; Popelar et al., 2006). In normal aging rats, a decrease in density of SGNs of 22% and 35% in the apical and basal turns, respectively, was seen in 20-24 month F344 rats (Buckiova et al., 2006) and IHC loss was typically under 10% (Popelar et al., 2006). These age-related changes are accompanied by smaller amplitude of later ABR waves at 12 and 20 months (Popelar et al., 2006) and a decrease in amplitude of the AP of the auditory nerve at 24 months (Bielefeld et al., 2008). Additionally, F344 rats show relatively small OHC loss (Popelar et al., 2003, 2006) but deterioration of OAEs due to disruption of prestin (Chen et al., 2009). Disruption of prestin would not impact CM amplitude due to the site of generation of the CM; and CM amplitude is not significantly diminished in prestin-knockout mice (Liberman et al., 2002). The study was terminated at 10 months of age in order to eliminate contribution of these age-related changes. Thresholds of right ears did not show significant differences after noise exposure that was consistent with age-related changes. Significant differences were detected in left ear thresholds between pre, 4 and 8 week thresholds and 24 week thresholds across frequencies that were most likely caused by elevation of 15 kHz thresholds in small number of animals. This suggests there may have been some age-related changes in this subset of animals at the end of the testing period.

Data collected from another group of rats (n=7) of the same ages that underwent the same EcochG testing protocol without exposure to any noise did not show any significant changes in the AP I/O functions or CM/AP ratio suggesting age-related changes did not play a major role in the changes measured by evoked potentials.

Lack of histological preparations at this time does not allow quantitative analysis of cochlear structures to be made. Until these analyses are completed, assumptions of loci of cochlear damage can only be made based on what is known about the generation sites of the components of EcochG. Additionally, data concerning the degree of TTS seen is limited and the level of exposure of the left ear after attenuation is not precisely known. Primary neural degeneration was previous only seen after a large TTS of approximately 40 dB (Kujawa & Liberman, 2009; Lin et al., 2011). This degree of degeneration may only occur after large threshold shifts, thus knowing the exact exposure and amount of threshold shift would help classify when primary neural degeneration may occur.

Due to the practical complications and variability in electrode impedance that can accompany recording non-transtympanic EcochG in humans, monitoring of the AP alone would not be effective since absolute amplitude would be expected to be variable based on electrode differences between testing sessions. Recording the CM/AP ratio allows for this variability to be accounted for and may help account for between test variability seen in the second group of animals that were exposed to a sub-clinical level. However, while this was the case for average data, many individual animal CM/AP ratios fluctuated over time while AP I/O functions were relatively stable. Using CM/AP ratio for chronic monitoring of integrity of SGNs clinically is limited due the individual fluctuation that was seen. However, with refinement of recording techniques and measurement of the

CM, this limitation may be overcome. Additionally, since CM was recorded without the use of high-pass masking noise, the resulting measures did not provide frequency specific information about the integrity of the OHCs over a large portion of the cochlea. Use of more frequency-specific recordings of the CM would allow for corresponding areas of the CM and AP to be assessed.

Chapter 5: Conclusion

Primary neural degeneration can occur in the mouse and guinea pig after a noise exposure that induced TTS of approximately 40 dB (Kujawa & Liberman, 2006, 2009; Lin, 2011). In this study, serial monitoring of AP I/O functions and the CM/AP ratio over a period of 24 weeks after narrowband noise exposure inducing a PTS or TTS was completed in order to assess if these components were sensitive to cochlear de-afferentation. In ears that experienced PTS or TTS, AP I/O functions displayed patterns consistent with cochlear de-afferentation. At four weeks post-noise exposure in ears that experienced TTS, AP I/O functions displayed a recruitment-like effect and athe CM/AP ratio was unchanged, suggesting underlying OHC pathology. In ears that experienced PTS, four weeks after noise exposure a large decrease in AP amplitude of approximately 70% was seen at all levels suggesting underlying OHC and IHC/neural pathology. The CM/CAP ratio was stable at this time due to the large reduction in both components. Subsequent reductions of AP amplitude were seen in ears that experienced PTS or TTS between 4 weeks and 8 weeks. The CM/AP increased with time due to the reductions in the AP suggesting cochlear de-afferentation. The findings indicate that components of EcochG are sensitive measures for assessment of de-afferentation of the cochlea after noise exposure.

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